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P.C. 2108.91

PATENT

#11 C. Griffin 6/10/91

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MAY 30 1991

GROUP 180

IN THE UNITED STATES PATENT & TRADEMARK OFFICE

In re application of LAWRENCE A. JOHNSON Method to Preselect the Sex of Offspring	)	Group Art Unit 188 Examiners J. Witz & D. W. Robinson

The Honorable

The Commissioner of Patents & Trademarks

Sir:

PRELIMINARY RESPONSE

Submitted herewith is a Declaration under 37 CFR 1.132 by applicant Johnson for the purpose of establishing the criticality of incubating sperm at temperatures ranging from 30°-39° C prior to sorting as required by the claims of record.

By way of introduction, semen collected for use in animal breeding operations are collected at body temperature, which in most mammals ranges from 36°-39° C. The semen is usually collected into a thermally protected insulated container that will allow the semen to cool gradually. The time required for it to reach room temperature is approximately a half hour or more. During this period, measurements are

taken to determine the volume, sperm count and percentage of motile sperm. The semen is then diluted with a specially designed diluent or extender that is species-specific and is designed to maintain the viability of the sperm during storage. If the semen is to be stored in the liquid state, it is cooled slowly by placing the semen which is in a water bath placed in a refrigerator. Over a period of several hours, the semen equilibrates to refrigeration temperature (approximately 5° C). It can be stored (depending on the species) at these conditions for several days and is used for insemination without prior warming. If the semen is to be frozen, it is cooled in the same manner, glycerol is added as a cryoprotectant, and freezing is conducted in liquid nitrogen vapor at a controlled rate from 5° C to about -120° C over approximately 10 minutes. This protocol will also vary somewhat for each species. After the semen reaches -120° C, it is plunged into liquid nitrogen for permanent storage at -196° C. Prior to insemination, the semen is thawed to about 15°-18° C. The warming of sperm in normal operations prior to insemination is avoided in order to maintain viability.

The fluorescent dyes that are used in the sorting method of the invention do not easily penetrate the plasma membrane surrounding the live sperm cell. When the cell ceases to be viable, then the membrane becomes

substantially more porous to the dye. However, the applicant has unexpectedly discovered that the plasma membrane can also be made more permeable to the dye if the sperm are incubated within the temperature range of 30°-39° C.

The Declaration Under 37 CFR 1.132 establishes the critical difference in dye absorbancy between sperm incubated at room temperature (22° C) and those incubated at 35° C. In Figure A, the preponderance of the sperm held for 15 min at room temperature absorb relatively little dye as indicated by the low level fluorescence (peak on the left). The few sperm which absorbed a large amount of dye (peak on the right) are no longer viable. Histograms B, C, and D illustrate the progressive shift from a large population of viable sperm to a population containing both viable and nonviable sperm as the time of room temperature incubation increases.

In Figure E, the sperm treated for 15 min at 35° C all absorb a relatively large amount of dye (high level of fluorescence). The single peak to the right of the histogram represents the combination of nonviable sperm and the sperm in which the plasma membrane porosity has been enhanced to be more open to the dye by virtue of the incubation temperature. The number of dead sperm is approximately the same as in the sample held at room temperature for 15 minutes. Figure F is the histogram of the sperm treated for 1 hr at 35° C. The two partially resolved peaks represent the male (Y) and female (X) sperm populations (viable and nonviable). Upon sorting, samples held for an hour or more at 35° C